

was refluxed for 2½ hr. with HBr (46–48%; 3 ml.). All the solid had then dissolved. On cooling, light greyish brown needles separated which still contained methoxyl (3.1%). The refluxing with HBr was continued for a further 1½ hr. Needle crystals (35 mg.; m.p. 232–234°) were obtained, of which the methoxyl content was nil. Recrystallization from ethanol-ether (1:1) with charcoal gave colourless needles (22 mg.) of the hydrobromide of 5:7-dihydroxy-6-methyl-phthalazine, m.p. 238–240° (decomp.), retaining ¼ mol. of ethanol even when dried at 100° in high vacuum [Found: C, 43.1; H, 4.2; N, 9.7; Br, 28.2; OEt, 7.9]. $C_9H_9O_2N_2Br$, ½ $C_9H_9 \cdot OH$ requires C, 42.9; H, 4.3; N, 10.0; Br, 28.5; OEt, 8.0%].

SUMMARY

1. *Aspergillus quadrilineatus* Thom & Raper, when grown on Raulin-Thom solution, produces in the culture medium a hitherto undescribed fungal metabolite, which has been named quadrilineatin.

2. Quadrilineatin, $C_{10}H_{10}O_4$, forms colourless needles of m.p. 172° (decomp.). From the study of its functional derivatives and oxidation products, a number of which are described, quadrilineatin has been allocated the structure 1:2-diformyl-5-hydroxy-3-methoxy-4-methylbenzene.

3. Quadrilineatin is thus a member of the group of substituted *o*-phthalaldehydes, of which three, namely gladiolic acid, cyclopaldic acid and flavipin, were previously known as fungal metabolites.

4. Quadrilineatin shows only weak antibacterial activity against *Staphylococcus aureus* and *Escherichia coli*.

The light-absorption values were determined by means of a Hilger Uvispek spectrophotometer purchased by means of a grant from the Central Research Fund of London University.

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APPENDIX

Anti-Fungal Tests on Quadrilineatin

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Quadrilineatin was tested by the method described by Brian & Hemming (1945) and used previously in this Laboratory for determining the fungistatic activity of cyclopaldic acid and related substances. The procedure is to determine the lowest concentration that will inhibit the germination of the conidia of *Botrytis allii* Munn. A series of twofold dilutions in 'germination medium' plus spore suspension were made, starting at 1 in 25 000. There was complete inhibition at the highest concentration only, 1:25 000 ($\equiv 40 \mu\text{g./ml.}$), partial inhibition up to 1:100 000 and virtually 100% germination at higher dilutions. The substance is therefore considerably less active than cyclopaldic acid, which showed complete inhibition at a dilution of 2.5 $\mu\text{g./}$

ml., and gladiolic acid and flavipin, which inhibited completely at 10 $\mu\text{g./ml.}$

A second series of tests were carried out to determine the activity of quadrilineatin against the 'damping-off' fungus *Pythium debaryanum* Hesse. A primary series of twofold dilutions in sterile water were made. Tubes containing exactly 14 ml. of Czapek-Dox agar were prepared and sterilized. To each of these, with the agar melted and then cooled to 45°, was added 1 ml. of aqueous solution. The primary dilutions were made so that on dilution 1:15 a series of dilutions in agar starting at 1:12 500 were obtained. The concentration required for the first of the primary dilutions was greater than the solubility of the substance in water. Complete

solution was effected by adding the least possible amount of sodium carbonate. The amount of alkali introduced in this way is too small to affect appreciably the pH of the strongly buffered Czapek-Dox agar.

The melted agar in the tubes was poured into the corresponding number of Petri dishes and allowed to set. Each dish was inoculated centrally with mycelium from a young culture of the *Pythium*. The results were recorded after 3 days' incubation, which is the time taken for a control plate to be completely covered. There was complete inhibition at a dilution of 1:25 000 and partial inhibition at 1:50 000. Thus two fungi belonging to widely different orders are inhibited completely by the same concentration.

SUMMARY

1. The inhibition of fungal growth by quadrilineatin was tested against two species of fungi. Complete inhibition of germination of *Botrytis allii* conidia and of mycelial growth of *Pythium debaryanum* occurred at a dilution of 1:25 000 (40 µg./ml.).

2. Quadrilineatin is a less potent inhibitor of *Botrytis allii* than the other known phthalaldehydes of fungal origin.

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The Flame-Spectrophotometric Determination of Calcium in Biological Fluids and an Isotopic Analysis of the Errors in the Kramer-Tisdall Procedure

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Although chemical methods have been superseded by the flame photometer in the determination of sodium and potassium in biological fluids, calcium is still usually determined chemically. The method in most widespread use is the procedure of Kramer & Tisdall (1921) or one of its modifications (Tisdall, 1923; Clark & Collip, 1925). This method involves the direct precipitation of calcium from serum with ammonium oxalate, isolation by centrifuging, and titration with permanganate after washing with dilute ammonia. This procedure is tedious and has other disadvantages.

Kirk (1954) regards the procedure as one of known unreliability. The same conclusion may be reached from a consideration of the conditions of precipitation which are far from optimum (Kolthoff & Sandell, 1952). Smith *et al.* (1950) showed that magnesium may be co-precipitated to the extent of 10% of the normal calcium content (expressed in equivalents) when serum is analysed. That such accuracy as the method possesses must depend on compensation of errors was well known to Clark & Collip (1925).

That flame photometry has not been widely used for calcium determination is due to two main difficulties. The first is the proximity of the re-

latively weak calcium oxide bands at 554 and 620 mµ to the intense sodium line at 590 mµ. This, together with the preponderance of sodium, makes marked spectral interference inevitable with the relatively simple instruments now in wide use (Powell, 1953). Even when instruments incorporating a monochromator (such as the Beckman flame photometer) are used, this difficulty is not entirely removed (Severinghaus & Ferrebee, 1950). Incorporation of sodium in the standard solutions (Baker, 1955) may allow correction to be made when serum calcium is being estimated, but the variability of sodium concentrations makes this procedure inaccurate when applied to urine.

The second difficulty is the fact that calcium is much more susceptible to interference from anions, in particular phosphate (Brealey, Garratt & Proctor, 1952; Chen & Toribara, 1953; Denson, 1954; Leyton, 1954; Baker & Johnson, 1954), than are sodium and potassium (Shapiro & Hoagland, 1948; Collins & Polkinhorne, 1952; Domingo & Klyne, 1949). As will be shown, neglect of this effect, as by Severinghaus & Ferrebee (1950), may cause serious error. Prior precipitation of calcium as oxalate (Powell, 1953; Llaurodo, 1954) should overcome both these difficulties; however, it leads only to combining some of the error of the oxalate procedure with little of the convenience of the flame photometer.

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